Nucleic Acid Footprinting of the Interaction of the TATA Binding Protein (TBP) with DNA

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The “TATA Binding Protein” (TBP) TBP is a protein required for the initiation of transcription by each of the three eukaryotic RNA polymerases. The binding of TBP to specific promoter sequences (“TATA boxes”) is a key step in the initiation of transcription of genes transcribed by RNA polymerase II. The application of synchrotron x-ray nucleic acid *and* protein footprinting to protein-DNA interactions is being addressed through the binding of TBP to a number of naturally occurring promoters. TBP binding induces a dramatic and drastic conformational change in the structure of the bound DNA with two kinks in the DNA stabilized by the intercalation of two pairs of phenylalanine residues. We have proposed that these intercalation events proceed sequentially and that their rates are DNA-sequence dependent. Synchrotron x-ray equilibrium titration experiments have been successfully conducted which demonstrate that accurate synchrotron footprinting data can be acquired for this system. As described elsewhere, quantitative relationships are present between the •OH protection pattern of a TBP-DNA complex and dynamics simulation. This correspondence will allow precise structural inferences to be drawn regarding the formation of the protein-DNA complex. Apparatus modification to allow protein-DNA kinetics experiments has been completed. Synchrotron footprinting TBP kinetics experiments that will critically test this hypothesis are first on the experimental queue once experiments are restarted after the spring beamline maintenance shutdown.